Activity of Garenoxacin (BMS284756) and Levofloxacin Against Intracellular *Staphylococcus aureus* or *Listeria monocytogenes* in J774 Macrophages

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ABSTRACT

Background:
Quinolones are active against a variety of intracellular organisms. Yet, little is known about the relationships between intrinsic activity (as determined in broth), cell accumulation, and intracellular activity.

Methods:
Mϕ were infected with serum-opsonized *S.a.* or untreated *L .m.* and exposed to increasing concentrations of GAR or LVX (in a clinically meaningful range) for 24 h (*S.*a.) or 5 h (*L.m.*). Activity in broth was measured in parallel. Cell accumulation of GAR and LVX (at equilibrium and recorded in distinct experiments) was approx 6-fold for GAR and 3-fold for LVX.

bacteria	Drug concentration (mg/L)	Activity (change in log CFU compared to time = 0)			
		in Mϕ		in broth	
		GAR	LVX	GAR	LVX
S.a.	0	1.50 ± 0.30		2.90 ± 0.10	
	0.12	-0.92 ± 0.03	-0.65 ± 0.28	-2.77 ± 0.03	2.83 ± 0.00
	0.5	-1.11 ± 0.16	-1.07 ± 0.03	-3.45 ± 0.06	-3.19 ± 0.04
	1	-1.27 ± 0.05	-1.28 ± 0.02	-3.79 ± 0.00	-3.75 ± 0.07
	4	-1.64 ± 0.16	-1.36 ± 0.05	-4.09 ± 0.00	-3.79 ± 0.00
L.m.	0	0.90 ± 0.10		1.23 ± 0.10	
	0.12	0.66 ± 0.02	0.80 ± 0.05	0.46 ± 0.03	1.20 ± 0.2
	0.5	-0.03 ± 0.04	0.76 ± 0.04	0.06 ± 0.01	0.47 ± 0.01
	1	-0.54 ± 0.05	0.66 ± 0.02	-1.30 ± 0.01	-0.23 ± 0.01
	4	-1.22 ± 0.06	-0.42 ± 0.09	-1.70 ± 0.02	-1.54 ± 0.02

Conclusion:
Both quinolones show a concentration-dependent intracellular activity, but the data suggest a marked defeating effect of the intracellular milieu on activity as compared to broth. For *L.m.*, the larger cellular accumulation of GAR and its higher activity in broth translated in a larger intracellular activity compared to LVX. This was not observed for *S.a.*, indicating that the relationship between activity in broth, cell accumulation and intracellular activity may vary according to the type of infection.

INTRODUCTION

- Eradication of intracellular infections requires the use of antibiotics able to accumulate in eucaryotic cells at sufficiently high concentrations.[1] Fluoroquinolones (zwitterions) accumulate in cells and could therefore be used against intracellular bacteria.[1,2]
- Garenoxacin (BMS-284756; formerly known is T-3811) is a novel des-fluoro (6)-quinolone more active against Gram-postive bacteria and intracellular organisms such as chlamydia.[3]

AIM OF THE STUDY

- To study the intracellular activity of garenoxacin compared to levofloxacin in a model of J774 mouse macrophages infected by a phagolysosomal bacteria *(Staphylococcus aureus)* or by a cytosolic bacteria *(Listeria monocytogenes).*
- To correlate intracellular activity with antibiotic cellular accumulation.

METHODS

Materials:
J774 macrophages, a continuous cell line derived from a mouse reticulosarcoma were maintained at 37°C, in a 5 % CO_2 atmosphere, in RPMI medium supplemented by 10 % foetal calf serum.

Levofloxacin [potency 99.7 %] was obtained from Aventis Pharma, Antony, France; garenoxacin [potency 99.8 %] from Bristol Myers Squibb, New Brunswick, CT, USA.

Cellular pharmacokinetics:

Cells were incubated for up to 24 h at 37°C with 5 µg/ml of the antibiotic. At the end of the incubation period, cells were washed 3 times in ice-cold PBS, collected in H_2O or glycine 0.1M, pH 3, and sonicated 10 sec at 50 W. Cell lysates were then used for measuring the cell protein content and the antibiotic concentration by an appropriate method (fluorimetric assay for levofloxacin and radiometric assay for garenoxacin). The apparent cellular accumulation (Cc/Ce) was calculated by the ratio of the drug cell content (Cc) to the drug extracellular concentration (Ce), assuming that 1 mg of protein corresponds to 5 µl of cellular volume.

Bacterial strain and determination of MICs and MBCs:

Hemolysin-producing strain EGD of *L. monocytogenes* (serotype 1/2a) and *S. aureus* ATCC25923 were used for all the experiments. MICs and MBCs were determined following the recommendations of the NCCLS.

METHODS (cont'd)

Activity against intracellular *Staphylococcus aureus* ATCC 25923:

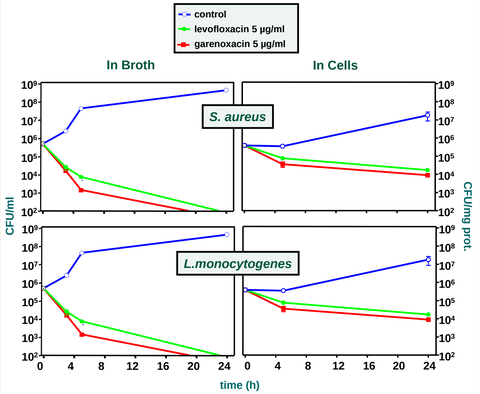
Bacteria were opsonized by human serum during 30 min. Cells were infected with an inoculum of 0.5 bacteria/macrophage, and washed during 1 h with gentamicin 50 µg/ml after 1 h of phagocytosis at 37°C to remove non-phagocytosed and non-firmly adherent bacteria. Cells were then incubated for up to 24 h with garenoxacin or levofloxacin or with gentamicin at its MIC (0.5 µg/ml; control).[4]

Activity against intracellular *Listeria monocytogenes*:

Bacteria were infected using an inoculum of 5 bacteria/macrophage, and washed with PBS after 1 h of phagocytosis at 37°C to remove non-phagocytosed and non-firmly adherent bacteria. Cells were incubated for 5 h with garenoxacin or levofloxacin.[5]

Viable bacteria were determined in cell lysates by colony counting (CFU).

RESULTS : pharmacodynamics

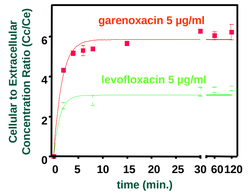


Left panel. Activity of garenoxacin and levofloxacin 5 µg/ml against *S. aureus* (top) or *L. monocytogenes* (bottom) in MH broth up to 24 h.

Right panel. Infected J774 macrophages were exposed to garenoxacin and levofloxacin for 24 hours at an extracellular concentration of 5 µg/ml.

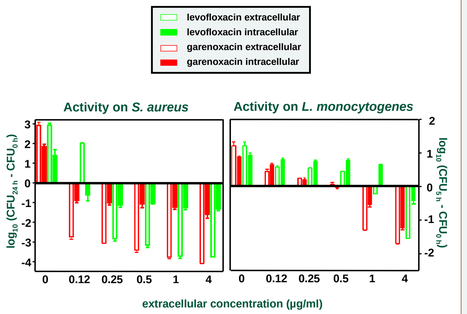
RESULTS : pharmacokinetics

Kinetics of Accumulation



Kinetics of accumulation of garenoxacin (red squares) and levofloxacin (green circles) in J774 murine macrophages incubated for up to 2 h with and extracellular concentration of 5 µg/ml. Results are expressed as the mean ± SD of three experiments.

RESULTS : pharmacodynamics



Variation in the number of CFU as compared to the initial inoculum after 24 h (*S.a.)* or 5 h (*L.m.*) of incubation in broth or in infected J774 macrophages with increasing concentrations of garenoxacin or levofloxacin (0.12, 0.25, 0.5, 1, 4 µg/ml).

0, control cells (no antibiotic in broth; for macrophages, control includes gentamicin [0.5 µg/ml] throughout the incubation period).

CONCLUSION

- Both quinolones show a concentration - dependent intracellular activity.
- Quinolone activity is lower intracellularly than extracellularly, suggesting a marked defeating effect of the intracellular milieu on activity as compared to broth.
- For *L. monocytogenes*, the larger cellular accumulation of garenoxacin and its higher activity in broth translate in a larger intracellular activity compared to levofloxacin.
- For *S. aureus*, garenoxacin and levofloxacin show a similar intracellular activity, despite the higher accumulation level of garenoxacin.
- The relationship between activity in broth, cell accumulation and intracellular activity may vary according to the type of infection.

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REFERENCES

1. Tulkens, P. M. 1991. Intracellular distribution and activity of antibiotics. Eur. J. Clin. Microbiol. Infect. Dis. 10:100-106.
2. Carlier, M. B., Scorneaux B., Zenebergh A., Desnottes J.F., and Tulkens P.M . 1990. Cellular uptake, localization and activity of fluoroquinolones in uninfected and infected macrophages. J. Antimicrob. Chemother. 26 Suppl B:27-39.
3. Fung-Tomc J.C., Minassian B., Kolek B., Huczko E., Aleksunes L., Stickle T., Washo T., Gradelski E., Valera L., Bonner D.P. 2000. Antibacterial spectrum of a novel des-fluoro(6) quinolone, BMS-284756. Antimicrob. Agents Chemother. 44: 3351-3356.
4. Seral, C., Van Bambeke, F., and Tulkens, P. M. 2003. Quantitative analysis of gentamicin, azithromycin, telithromycin, ciprofloxacin, moxifloxacin, and oritavancin (LY333328) activities against intracellular *Staphylococcus aureus* in mouse J774 macrophages. Antimicrob.Agents Chemother. 46: 2095-2103.
5. Carryn, S., Van Bambeke, F., Mingeot-Leclercq, M. P., and Tulkens, P. M. 2002. Comparative intracellular (THP-1 macrophages) and extracellular activities of beta-lactams, azithromycin, gentamicin, and fluoroquinolones against *Listeria monocytogenes* at clinically relevant concentrations. Antimicrob. Agents Chemother. 46: 2095-2103.